INHIBITION OF BISPECIFIC MONOCLONAL ANTIBODY (bsAb)-TARGETED CYTOLYSIS BY HUMAN ANTI-MOUSE ANTIBODIES IN OVARIAN CARCINOMA PATIENTS TREATED WITH bsAb-TARGETED ACTIVATED T-LYMPHOCYTES

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T lymphocytes of 8 patients with ovarian cancer were targeted to the tumor cells using F(ab')2 fragments of a bispecific monoclonal antibody (bsAb), specific for CD3 (a component of the T lymphocyte receptor for antigen) and for the folate receptor MOv18 (overexpressed by ovarian carcinoma cells) as part of a phase I/II study. Phase I (days 0 to 3) consisted of increasing intraperitoneal (i.p.) numbers (104 to 109) of bsAb-targeted lymphocytes plus low-dose interleukin-2 (IL-2). Phase II (days 6 to 13, and 27 to 33) consisted of daily i.p. infusions of 109 targeted T lymphocytes, 2 mg solubilized bsAb, and low-dose IL-2. Using enzyme-linked immunosorbent assays (ELISA), human anti-mouse antibodies (HAMA) were detected in all patients: in the serum from day 13 onwards and in the peritoneal fluid from day 20 onwards. A significant proportion of the HAMA appeared to be directed against the idiotypes of the bsAb specific for CD3 and MOv18, as suggested by (1) the clearly higher ELISA titer against OC/TR bsAb as compared to those against a non-monoclonal antibody (MAb) with unrelated specificity, and (2) failure to abrogate the capacity of human anti-mouse IgG-framework antibodies in peritoneal fluid to immobilize T lymphocytes that were inhibited by peritoneal fluid samples containing relatively high HAMA titers. Such inhibitory activity was never detected at the start of phase II, but coincided with the last series of i.p. infusions of targeted T lymphocytes in 2 patients.

Mouse MAbS directed against tumor-associated antigens (TAA) and conjugated to radioisotopes, chemotherapeutic agents or toxins are increasingly being used for in vivo diagnosis and treatment of malignancies (Mach et al., 1981; Schroff et al., 1985). The infusion of murine immunoglobulins (Ig) elicits human anti-mouse Ig antibody (HAMA) responses. Such HAMA responses have been reported in clinical studies of tumor imaging (Courtenay-Luck et al., 1986) and tumor therapy (Schroff et al., 1985;Courtesy-Luck et al., 1988; Saleh et al., 1993). HAMA responses develop over a wide dose range (i.e., from <1 mg to >1 g) (Khazaie et al., 1988), and occur more frequently after repeated administrations of MAbS (Lind et al., 1991). Fab fragments of murine MAbS and genetically engineered mouse-human MAbS are less immunogenic than intact murine Ig molecules (reviewed by Khazaie et al., 1994). Initially, HAMA with specificities for the constant regions of murine Ig develop, followed by HAMA with anti-idiotypic specificities. These latter antibodies develop particularly well after multiple administrations of MAbS (Schroff et al., 1985; Saleh et al., 1993). Adverse clinical reactions upon administration of MAbS to patients with pre-existing HAMA are uncommon, but a highly increased clearance of the administered MAb is seen in such patients, which may prevent the MAbS from interacting with their target (Khazaie et al., 1994).

Targeting of the cytolytic activity of T lymphocytes against tumor cells can be achieved using bispecific monoclonal antibodies (bsAbS) which are directed against a T-cell activation epitope on the one hand (e.g., CD3) and a TAA on the other. Targeting of cytotoxic T lymphocytes (CTL) with bsAb results in selective destruction of tumor cells following MHC-unrestricted binding of the CTL to tumor cells (Segal and Snider, 1989; Van Dijk et al., 1989). This approach has been effective in reducing or eliminating tumor in several mouse model systems (Segal and Snider, 1989), in human malignant glioma (Nitta et al., 1990) and in ovarian carcinoma (Bolhuis et al., 1992). The OC/TR bsAb is specific for CD3 and MOv18 (Mezzanzanica et al., 1988). MOv18 has been identified as the glycosyl-phosphatidyl-inositol (GPI)-anchored, high-affinity folate-binding protein gp38 (Coney et al., 1991), and is overexpressed on ovarian carcinoma cells as compared to normal ovarian cells. The OC/TR bsAb was used as part of the intraperitoneal (i.p.) treatment of ovarian carcinoma in a phase-I/II clinical trial (Bolhuis et al., 1992). Patients entered into this trial received 10 therapeutic i.p. infusions with OC/TR and autologous OC/TR-targeted activated T lymphocytes. This report concerns the kinetics of HAMA responses in these patients and the interference of HAMA with bsAb-targeted cytosis.

MATERIAL AND METHODS

Clinical study

Eight ovarian-carcinoma patients were treated with i.p. infusions of targeted T lymphocytes and the bsAb OC/TR in a phase-I/II clinical trial. Patients with advanced epithelial ovarian carcinoma (FIGO stage III, MOv18-positive tumor, and thickness of residual tumor lesions of less than 2 cm after debulking surgery), were eligible. T lymphocytes were activated in vitro and expanded using phytohemagglutinin (PHA; Wellcome, Dartford, UK) and interleukin-2 (IL-2; Proleukin, Eurocetus, Amsterdam, The Netherlands) as described previously (Lamers et al., 1992). The bsAb OC/TR F(ab')2 fragments were produced by Centocor Europe (Leiden, The Netherlands). Prior to i.p. administration, the cultured T lymphocytes were incubated for 30 min at 37°C with OC/TR bsAb (1 mg per 109 viable cells; Table I). Thereafter, unbound bsAb was washed away in phase I but was not removed in phase II, and 0.6 x 106 IU IL-2 were added prior to administration in all cases. According to the schedule (Table I) the patients were to receive 3 cycles of i.p. therapy of autologous OC/TR-labeled T lymphocytes, soluble OC/TR and IL-2. Thus, they should have received a total of 10 x 106 OC/TR-labeled T lymphocytes, 28 mg OC/TR bsAb and 16.8 x 106 IU IL-2 during phase II. The actual treatment differed from schedule in 3 of the 8 patients. Patient 2 received a third therapeutic cycle from days 48 to 52 because her first therapeutic cycle was considered inadequate due to insufficient fluid distribution in the peritoneal cavity. For patient 6,

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the interval between phase I and the first therapeutic cycle of phase II was extended to 14 days because of slow in vitro expansion of the autologous T lymphocytes. Treatment of patient 8 was aborted on day 28 (i.e., after the first day of the second therapeutic cycle) due to her poor clinical condition.

Serum and peritoneal fluid samples were taken prior to the first infusion and on days 6, 13, 27, 34, 49, and around days 70 and 100. Serum and peritoneal fluid samples were cleared from cells and debris by centrifugation and stored at −80°C until further investigation.

Detection of human anti-mouse antibodies (HAMA)

The presence of HAMA in serum and peritoneal fluid samples was assessed using a 2-step sandwich enzyme-linked immunosorbent assay (ELISA). As solid phase, F(ab′)2 fragments of the OC/TR bsAb (IgG1/IgG2), of the 323/A3 MAb (an IgG1, κ MAb with pan-carcinoma specificity; Edwards et al., 1986) and of the MOv18 MAb (IgG1, κ were used (all from Centocor Europe, Leiden). The tracer antibody was goat anti-human Fc(γG) conjugated with alkaline phosphatase (Affipure; Jackson Immunotech, West Grove, PA) and the substrate was p-nitrophenylphosphate disodium dissolved in 2-amino-2-methyl-1-propanol (Sigma, St. Louis, MO) at pH 10.3. The wells of 96-well microtiter plates (Nunc, Rosknidle, Denmark) were coated with 2 μg of antibody in 50 μl of 0.1 M glycine, 0.5 mM EDTA, pH 8.5, for 16 hr at 4°C. After washing with PBS (pH 7.4), free binding sites on the matrix were blocked with 150 μl PBS containing 1% BSA, for 1 hr at 37°C. The assay samples (80 μl/well, diluted in PBS + 1% BSA) were incubated for 2 hr at 37°C. The initial dilution was 1:20. A HAMA-positive serum and a pool of normal human sera were run with each assay plate as positive and negative controls, respectively. The plates were washed 5 times with PBS containing 0.1% Tween-20 and incubated with 75 μl/well of tracer antibody (diluted 1:2,000 in PBS + 1% BSA) for 1 hr at 37°C. After washing with PBS + 0.1% Tween-20, the plates were incubated with 100 μl/well substrate for 30 min at 37°C, and the reaction was abrogated by adding 50 μl 3N NaOH. The optical density (OD) was read at 405 nm. The reciprocal dilution of the serum sample with OD < 0.1 was arbitrarily referred to as the HAMA titer.

Removal of human antibodies to murine IgG from peritoneal fluid

Human anti-murine IgG antibodies were removed from sera and peritoneal fluid by affinity absorption using mouse IgG (Sigma) coupled to Affi-gel 10 (BioRad, Richmond, CA). For coupling, 10 mg mouse IgG in 0.5 ml 1 M NaCl, 80 mM CaCl2 and 0.1 M HEPES (pH 7.2), was incubated with 1 ml Affi-gel (10 mg/ml) for 1 hr at 37°C. After washing with PBS + 0.1% Tween-20, the plates were incubated with 100 μl/well substrate for 30 min at 37°C, and the reaction was abrogated by adding 50 μl 3N NaOH. The optical density (OD) was read at 405 nm. The reciprocal dilution of the serum sample with OD = 1.0 was arbitrarily referred to as the HAMA titer.

Flow cytometric assay of HAMA-mediated inhibition of OC/TR bsAb binding to Igrov-1 and D11 cells

The MOv18* ovarian carcinoma-derived cell line Igrov-1 (Bérard et al., 1985) and the CD3+, 4+, 8+, TCRβ+ CTL clone D11 (Van de Griend et al., 1984) were used to analyze HAMA-mediated inhibition of binding of OC/TR bsAb to these cells. From each cell line, 50×10⁶ cells were incubated for 30 min at 4°C with 50 μl of (1) PBS + 1% BSA; (2) 1 μg/ml OC/TR F(ab′)2; (3) human anti-idiotypic MAb (A.B.C MAb obtained from SeraLab, Crawley Down, UK) diluted in PBS + 1% BSA; (4) HAMA containing peritoneal fluid (diluted 1:2 in PBS + 1% BSA); (5) same as (4), with OC/TR bsAb; and (6) same as (4), with W6/32 MAb added 30 min prior to the assay. Thereafter, the cells were washed once and incubated with goat anti-mouse Ig conjugated with phycoerythrin (PE; Southern Biotechnology, Burlington, VA) for another 30 min at 0°C. After washing once, the cells were fixed in PBS containing 10 mg/ml paraformaldehyde and analyzed using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). For data acquisition, list mode data of 10,000 cells per sample were obtained. In the analyses of those data, dead cells and cell fragments were excluded on the basis of their low forward light scatter signals, after which PE fluorescence characteristics were studied using histogram statistics.

Cytotoxicity (CTX) assay

Cytolytic activities were assessed in a standard 3-hr ⁵¹Cr-release assay, using T lymphocytes from ovarian cancer patients, activated and expanded using PHA + IL-2, and the CTL clone D11, specific for HLA-Cw3, as effector cells (Bolhuis and Van de Griend, 1985). The ovarian carcinoma cell line Iov-1 and the Epstein-Barr virus-transformed, HLA-Cw3+ lymphoblastoid cell line BSM served as the source of target cells. The CTX assay medium was RPMI-1640 supplemented with 10% newborn bovine calf serum (GIBCO, Grand Island, NY). The effector cells were pre-incubated with OC/TR for 30 min at 37°C, 2% FCS/ml of OC/TR, was sufficient for optimal targeting of cloned and bulk-cultured CTL (Mezzanzanica et al., 1988). In one experiment, chimeric F(ab′)2; OC/TR (chOC/TR; Centocor Europe), in which the murine constant part of the antibody was replaced by the corresponding human constant part, was used to target D11 cells. Varying numbers of effector lymphocytes were seeded in triplicate in 96-well, round-bottomed microtiter plates (100 μl/well), followed by 2,500 target cells in 100 μl per well. The target cells were labeled with 100 μCi ⁵¹Cr per 0.5 × 10⁶ cells for 2 hr at 37°C. The effector to target (E:T) ratios used in most cases were 50, 25, 12 and 6:1. At the end of the 3-hr incubation period (37°C and 5% CO₂), supernatants were collected and ⁵¹Cr release was measured in a gamma-counter. The percentage of specific lysis was calculated according to standard formulas. The data were expressed as percentage specific lysis for each of the 4 E:T ratios tested.

For analysis of the interference of HAMA in peritoneal fluid with in vitro OC/TR-mediated cytolytic activity, autologous T lymphocytes that had been activated and expanded using PHA and IL-2 were targeted with OC/TR F(ab′)2, as described above, and subsequently washed twice to remove unbound OC/TR. OC/TR-targeted or untargeted T lymphocytes were resuspended in undiluted, heat-inactivated (30 min at 56°C) peritoneal fluid samples. The target cells were resuspended in the corresponding peritoneal fluid samples. A similar CTX assay performed in CTX assay medium instead of peritoneal fluid served as positive control in each experiment, whilst CTX assays using untargeted T lymphocytes served as negative controls.

RESULTS

HAMA in serum and peritoneal fluid become detectable during therapy and contain anti-idiotypic antibodies

HAMA became detectable in the serum and peritoneal fluid of all 8 patients during treatment (Fig. 1). Serum IgG antibodies reactive with OC/TR bsAb were first detectable after a median of 20 days (range 13 to 27) and reached their peak after a median of 49 days (range 27 to 70) after the start of phase I. The median peak serum titer was 107,000 (range 51 to 1.4 × 10⁸) per ml. Serum IgG antibodies reactive with W6/32 showed similar kinetics but reached lower titers (median peak serum titer, 12,000 [range 50 to 45,000]).
FIGURE 1 - HAMA titers in sequential serum and peritoneal fluid samples from 8 patients. For each patient, serum titers are set out in the left panel and peritoneal fluid titers in the right panel. HAMA titers against OC/TR bsAb are represented by closed symbols connected by bold lines; those against 323/A3 MAb (with specificity unrelated to that of OC/TR) by open symbols connected by thin lines. Hatched boxes represent time periods during which immunotherapy (1 phase-I cycle, followed by 2 to 3 phase-II cycles) was administered. Logarithmic scales were used for the Y axes in order to compress the figures.
that a significant component of HAMA was directed against the idiotypes of OC/TR bsAb.

The kinetics of IgG antibody titers reactive with OC/TR bsAb or 323/A3 MAb in peritoneal fluid paralleled those in the serum. HAMA reactive with OC/TR bsAb were first detectable after a median of 22 days (range 20 to 34) and reached their peak after a median of 48 days (range 27 to 70) after the start of phase I. The median peak titer was 27,000 (range 200 to 1.6 × 10^5). As in the serum, the titers of HAMA reactive with 323/A3 were lower than those reactive with OC/TR bsAb (median peak titer, 1,075 [range 45 to 50,000]). The fluctuations of HAMA titers in the peritoneal fluid observed in patients 2, 4 and 5 may have been caused in part by dilution by the i.p. infusions of targeted T lymphocytes (2 × 500 ml daily) and by the rinsing of the peritoneal cavity with saline between the 2 therapeutic cycles.

Further evidence that HAMA by and large comprised anti-idiotypic antibodies reactive with OC/TR bsAb was obtained by the observation that removal of human anti-mouse IgG antibodies by absorption to murine IgG coupled to Affigel 10 from the day-48 peritoneal fluid sample of patient 2 did not abrogate its capacity to block the binding of OC/TR bsAb to Igrov-1 ovarian carcinoma cells or to the CTL clone D11. The 2-fold absorption procedure of this specimen with mouse IgG coupled to Affigel-10 led to a 98% reduction in titer of antibodies reactive with 323/A3 MAb (i.e., from 4,200 to 70), whilst only a 43% reduction in titer of antibodies reactive with OC/TR bsAb (median peak titer, 1,075 [range 45 to 50,000]). The fluctuations of HAMA titers in the peritoneal fluid observed in patients 2, 4 and 5 may have been caused in part by dilution by the i.p. infusions of targeted T lymphocytes (2 × 500 ml daily) and by the rinsing of the peritoneal cavity with saline between the 2 therapeutic cycles.

Interaction between HAMA and OC/TR-targeted cytolyis of Igrov-1 ovarian carcinoma cells

Since MOv18 is shed, although to a limited extent, from the surface of fresh ovarian tumor cells and cell lines (Miotti et al., 1992), ascitic or peritoneal fluid samples from such patients may contain free MOv18 glycoproteins which may interfere with in vitro assays of OC/TR-targeted cytolyis. However, we observed that the levels of bsAb-targeted cytolyis of Igrov-1 cells in the presence of peritoneal fluid obtained prior to treatment (day 0) were similar to those in the presence of standard assay medium in all of the 7 patients investigated in this respect (Fig. 3).

Next, we studied whether or not peritoneal fluid samples containing HAMA could inhibit OC/TR-targeted cytolyis of Igrov-1 cells. Inhibition was detected in peritoneal fluid samples of 6 of the 8 patients, and particularly in samples with relatively high HAMA titers (Fig. 3). Seven of 8 peritoneal fluid samples with HAMA titers of 5,778 or more inhibited OC/TR-targeted cytolyis of Igrov-1 cells vs. only 1 of 20 samples with HAMA titers <5,778 (Fisher's exact test, p < 0.001). Inhibition was only detected after completion of phase II in 4 patients (1, 4, 6 and 7), but coincided with therapy.
Figure 3 - Interaction between HAMA in sequential peritoneal fluid samples and OC/TR-targeted cytolysis of Igrov-1 ovarian carcinoma cells by autologous T lymphocytes activated and expanded using PHA + IL-2. The sampling time point of each peritoneal fluid sample is indicated as day relative to start of phase I. Arrows indicate the end of phase II. The HAMA titer against OC/TR bsAb (ELISA assay) is indicated between brackets for each peritoneal fluid sample. CTX assays using OC/TR-targeted T lymphocytes as effector cells are represented by closed symbols connected by bold lines; the corresponding assays using untargeted T lymphocytes are represented by open symbols connected by thin lines. CTX assays performed in CTX assay medium (indicated by "Med") instead of peritoneal fluid served as positive controls for OC/TR-targeted cytolysis of Igrov-1 cells in each experiment. The E:T ratios in assays designated 3–25 were 3, 6, 12 and 25:1, respectively; those in assays designated 6–50 were 6, 12, 25 and 50, respectively. All peritoneal fluid samples were heat-inactivated (30 min at 56°C) prior to the CTX assays.
in 2 patients (2 and 5). Patient 2 received a third therapeutic cycle (days 48–52). Peritoneal fluid obtained at days 48 and 54 completely inhibited OC/TR-targeted cytolysis of Igrov-1 cells. The day-27 sample of patient 5, obtained immediately before the start of the second phase-II therapeutic cycle (Table I), also inhibited OC/TR-targeted cytolysis of Igrov-1 cells. This sample had an exceptionally high HAMA titer (i.e., ranging between 20 and 267; patients 1, 2, 6, 7 and 8). The increased lysis of Igrov-1 target cells by untargeted T lymphocytes in patient 1 (days 27 and 34), patient 4 (days 13), patient 5 (days 13 and 34), patient 6 (day 27), patient 7 (days 13 and 27) and patient 8 (days 13 and 30) was caused by the presence of free OC/TR bsAb in those peritoneal fluid samples. This presence was detected by incubation of D11 and Igrov-1 cells in peritoneal fluid followed by indirect immunofluorescence (data not shown).

Finally, we analyzed the interaction between HAMA in peritoneal fluid samples, and (1) OC/TR-targeted cytolysis of Igrov-1 cells; (2) chOC/TR-targeted cytolysis of Igrov-1 cells, and (3) untargeted, HLA-Cw3-specific cytolysis of BSM cells, by D11 effector cells (Fig. 4). Three peritoneal fluid specimens were selected: one from patient 1, obtained on day 69 (HAMA titers [ELISA assay] against OC/TR bsAb, 896,000; against 323/A3 MAb, 17,000); one from patient 2, obtained on day 48 and diluted 1:4 in CTX medium (final HAMA titers 2,093 and 236, respectively); and one from patient 5, obtained on day 48 (HAMA titers 193,400 and 23,500, respectively). First, all 3 samples completely inhibited OC/TR-targeted cytolysis of Igrov-1 cells by the CTL clone D11 (Fig. 4, upper panel). A combination of human anti-idiotypic and anti-murine Ig framework antibodies may be responsible for this inhibition. Second, the possible inhibitory effect of HAMA against the murine C11 and C1 domains was excluded in the subsequent assay in which the D11 cells were targeted using chOC/TR (Fig. 4, middle panel). The cytolysis of Igrov-1 cells by chOC/TR-targeted D11 cells was still completely inhibited in the assays with the highest HAMA concentrations, i.e., those carried out using the peritoneal fluid samples of patients 1 and 5, whilst partial inhibition was seen in the assay with the lowest HAMA concentration, i.e., the one carried out in the 1:4 diluted peritoneal fluid from patient 2. This observation indicates that the inhibition of OC/TR-targeted cytolytic activity by patient 2’s peritoneal fluid was partly due to anti-murine C11 and/or C1 antibodies. Third, HAMA had no effect on untargeted, HLA-Cw3-specific cytolysis of BSM cells by the D11 effector cells (Fig. 4, lower panel).

**DISCUSSION**

We have studied the kinetics of HAMA responses and the interference of HAMA with OC/TR bsAb-targeted cytolysis  

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<td>I</td>
<td>0</td>
<td>10^6 Ly × OC/TR^2 + IL2</td>
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<td>1</td>
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<td>3</td>
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<td>11, 12</td>
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<td>II (2nd therapeutic cycle)</td>
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<td>32, 33</td>
<td>IL2 + OC/TR</td>
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The 1st infusion was administered at 12 a.m. and the 2nd infusion at 8 p.m. *Unbound OC/TR, autologous T lymphocytes targeted with OC/TR bsAb; IL-2, 0.6 × 10^6 IU/IL-2; OC/TR, 1 mg soluble OC/TR bsAb. All infusions were given in 500 ml of Ringer’s lactate solution supplemented with 1% human serum albumin.* Unbound OC/TR bsAb was washed away. Unbound OC/TR bsAb was not removed.

**FIGURE 4** Interaction between HAMA in peritoneal fluid and OC/TR-targeted (upper panel), chOC/TR-targeted (central panel) and HLA-Cw3-specific (lower panel) lysis by D11 CTL. The following peritoneal fluid samples were used: patient 1, day 69 (HAMA titers [ELISA assay] against OC/TR bsAb, 896,000; against 323/A3 MAb, 17,000); patient 2, day 48, 1:4 diluted (HAMA titers after 1:4 dilution, 2,093 and 236, respectively); and patient 5, day 48 (HAMA titers 193,400 and 23,500, respectively). CTX assays using OC/TR-targeted or chOC/TR-targeted D11 CTL are represented by open symbols connected by solid lines; assays using untargeted D11 CTL are represented by solid symbols connected by bold lines. CTX assays performed in CTX assay medium (indicated by “Medium”) instead of peritoneal fluid served as positive controls in each experiment. The E:T ratios were 3, 6, 12 and 25:1, respectively. All peritoneal fluid samples were heat-inactivated (30 min at 56°C) prior to the CTX assays.

in 8 ovarian carcinoma patients receiving phase-I/II i.p. immunotherapy using OC/TR-targeted lymphocytes, soluble OC/TR bsAb and low-dose IL-2. HAMA were detected using ELISA in serum and peritoneal fluid samples of all 8 patients from 2 and 3 weeks following start of therapy onwards, respectively, and reached peak levels at 7 weeks. These results are consistent with published data on the serum kinetics of
primary IgG HAMA responses following intravenous (Courtenay-Luck et al., 1986) or intraperitoneal (Maher et al., 1992) administrations. A significant part of the HAMA appeared to be directed against the idiotypes of OC/TR bsAb specific for CD3 and MOv18, as suggested by (1) the clearly higher ELISA titers against OC/TR bsAb compared to those against a MAb with unrelated specificity, and (2) the failure to abrogate the capacity of peritoneal fluid to block the binding of OC/TR bsAb to MOv18+ or CD3+ cells by absorption of human anti-mouse IgG-framework antibodies in peritoneal fluid to immobilized mouse IgG. Indeed, anti-idiotypic HAMA responses have been observed in particular after repeated MAb administrations (Schroff et al., 1985; Courtenay-Luck et al., 1988; Saleh et al., 1993).

The specificity of HAMA for the complementarity-determining region (CDR) of MAb has been demonstrated previously using inhibition of antigen binding (Traub et al., 1988) or inhibition of anti-idiotypic antibody binding to that MAb (Chattopadhyay et al., 1991). We have followed the first approach (Fig. 2). An important observation was that peritoneal fluid samples with relatively high HAMA titers inhibit in vitro OC/TR-targeted cytolyis of ovarian carcinoma cells (Fig. 3). This inhibitory activity was never detected at the start of the therapeutic infusions of OC/TR-targeted T lymphocytes (i.e., phase II), but became detectable during phase II in 2 patients. This result suggests that HAMA may interfere with the goal of therapy, i.e., the in vivo destruction of tumor cells by bsAb-targeted T lymphocytes.

**REFERENCES**


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**Strategies for overcoming the immunogenicity problem**

include the use of murine–human antibody constructs: chimeric MAb, composed of murine Vg and Vh regions combined with human CH1 and CH2 regions, or CDR-grafted MAb, which consist not only of human constant regions but also of the framework of human Vg and Vh regions (reviewed by Khazaeli et al., 1994). Although such molecules are probably less immunogenic than their entirely murine counterparts, they still elicit anti-idiotypic HAMA responses and, hence, antibodies with different anti-tumor idiotypes need to be used in the case of prolonged or repeated therapeutic interventions using bsAb-targeted T lymphocytes. An alternative to the use of autologous bsAb-targeted T lymphocytes is offered by genetically engineered T lymphocytes endowed with receptors specific for tumor-associated antigens (Hwu et al., 1993), which may be less immunogenic.


